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PHYSIOLOGICAL STUDIES ON THE THERMOPHILIC FUNGUS *TALAROMYCES THERMOPHILUS* STOLK

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ABSTRACT

The thermophilic fungus *Talaromyces thermophilus* (conidial state, *Penicillium dupontii*) was found to produce extracellular cellulase, amylase, and pectinase when grown in liquid culture on media containing different carbon sources. The type of carbon source had a marked influence on enzyme production. Enzymic activity was greater at 50°C than at 25°C.

INTRODUCTION

Thermophilic fungi comprise a limited number of species which have the ability to grow at temperatures above those considered to be the maximum (40°C) for most fungi. The abundance of these organisms in self-heating piles of organic matter has caused concern because of their contribution to biodeterioration and spontaneous combustion of stored agricultural and forest products, and because they can cause diseases of man and other animals.

Cooney and Emerson (1964), in their authoritative account of the thermophilic fungi, defined them as having a maximum temperature for growth at or exceeding 50°C and a minimum temperature for growth at or above 20°C.

In view of the intensive investigations that have been made of thermophilic bacteria and algae, the paucity of knowledge of the thermophilic fungi is surprising. Standard mycological texts pay little or no attention to them. The small amount of work done has, in the main, been devoted to morphological, systematic and cultural studies. The physiology of the thermophilic fungi is a sadly neglected field.

During decomposition of organic matter, as in composting, it has been assumed that thermophilic micro-organisms are important in the breakdown of the constituent materials. However, the exact role of thermophiles in the decomposition of such organic matter has not been clarified. There have been numerous reports in the literature (for example, Fergus 1969a) that these fungi possess cellulolytic activity but because of difficulties in species identification and the variable criteria used for assessing cellulase activity many of these reports are contradictory.

Thermophilic fungi are most frequently isolated from composting plant material which contains cellulose, hemicelluloses, pectins and starch as potential nutrients. An investigation of the ability of thermophilic fungi to hydrolyze these compounds

would aid in elucidating the role they play in biodegradation and could suggest potential applications for the fungi in industrial enzyme processes.

The growth, cellulolytic, pectolytic and amylolytic activity of the Penicillium state of the thermophilic fungus *Talaromyces thermophilus* (Stolk 1965) has been investigated and is reported here.

MATERIALS AND METHODS

T. thermophilus was isolated on yeast-glucose agar (YG) from compost material obtained from the botanic garden, University of Canterbury, and incubated at 50°C.

GROWTH EXPERIMENTS

Inoculum for growth experiments was obtained from cultures grown on yeast-starch (YS) agar plates (Emerson 1941). A sharpened, sterile, 5 mm cork borer was used to cut agar discs from the growing edge of the fungal colony. The discs were inverted and placed at the centre of 90 mm plastic petri dishes containing 30 ml YS agar and the dishes were incubated at 20, 25, 30, 50, 60 and 65°C. Cultures were examined after seven days and colony diameters were measured twice, at right angles to one another, and averaged to give an average colony diameter for each dish.

EXTRACELLULAR ENZYME PRODUCTION

The fungus was grown in stationary liquid culture on a medium containing:- Casein hydrolysate (0.4%); KH_2PO_4 (0.1%); $MgSO_4.7H_2O$ (0.05%); 10 ml trace element solution $FeSO_4.7H_2O$ (0.02 g) $ZnSO_4.2H_2O$ (0.1 g); $CuSO_4.5H_2O$ (0.02 g); $NaMoO_4.2H_2O$ (0.002 g); $MnCl_2.4H_2O$ (0.002 g) and a main carbon source (1%) in a total volume of 1000 ml.

Four different main carbon sources were used:- glucose, carboxymethylcellulose (CMC), pectin and sodium polypectate (NaPP). The pH of the culture media was adjusted to pH 5.0 and checked after autoclaving.

Twenty-five ml aliquots of medium containing each of the carbon sources were dispensed into 'medical flat' bottles ('16 oz' flats), inoculated with an 8 mm diameter agar disc taken from the growing edge of a culture on YS agar, and incubated at 50°C for 7 days.

Cultures were filtered through Whatman No. 1 filter paper, centrifuged at 10,000x g for 20 minutes to render cell-free, and stored in McCartney bottles at -20°C until required.

ENZYME ASSAYS

1. Cup-Plate Assay

The 'cup-plate' assay procedure of Dingle *et al.* (1953) was used to estimate amylase, cellulase and pectinase activity. In plates containing either soluble starch, carboxymethyl cellulose or sodium polypectate in a 2% agar solution buffered at pH 4.5,

8 mm diameter cups were cut and partially filled with culture filtrate. A coverslip was placed on top of each cup to minimise evaporation. Duplicate sets of plates were incubated at 25°C and 50°C for 48 h. Control enzyme solutions, heated to 100°C for 10 min., were treated in an identical manner.

The plates were developed as follows:

Amylase:- 0.1N-Iodine solution to give colourless zones on a blue background.
Cellulase:- 10% (w/v) solution of copper acetate to give cloudy zones on a clear background.
Pectinase:- 5N-HCl to give an opalescent halo.

The diameter of the zones of hydrolysis were measured. The relationship between zone diameter and enzymic activity is linear over a wide range (Dingle *et al.* 1953).

2. Agar-Diffusion Assay for Cellulolytic Activity

This method is that of Rautela and Cowling (1966) designed to assay ability to degrade acid-swollen cellulose. Active cellylolytic fungi growing on the surface of an agar column which contains acid-swollen cellulose particules secrete enzymes which diffuse into the agar, dissolving the particles and forming a clear zone.

The assay medium and acid-swollen cellulose were prepared as described by Tansey (1971). The medium was dispensed into 'universal' bottles fitted with screw caps, each bottle containing a 40 mm column of medium. The pH of the medium after autoclaving was 6.4.

Cultures for inoculum were grown at 50°C on YS agar and inoculated onto the assay medium and onto control slants of carrying media. Depth of clearing was measured in mm, with the tubes illuminated from the side and against a black background.

RESULTS

Penicillium dupontii

Isolation of this species was made on YG agar. Growth was rapid at 50°C (Fig. 1). The colony reached a diameter of about 80 mm in 7 days. The mycelium was described by Cooney and Emerson (1964) as delicately floccose, becoming somewhat mealy in older cultures, 1 mm deep or less, and in old age developing deeper tufts as over-growths. Dark brown drops of liquid exudate were sometimes formed in the central areas of agar cultures. Depending upon temperature, age, and substratum, the colours of the mycelium vary considerably. On YG agar at 50°C the growth was always white initially, and then became a rather dull shade of greyish-green, lavender, or pinkish-brown. Conidiophores (Fig. 2) developed as short lateral branches more or less perpendicular to the main hyphae from which they arose. Irregular branches, with one to four phialides at the apex of a short conidiophore, were observed. Conidia were borne in long tangled chains on the phialides. Mature conidia were smooth, and generally elliptical or slightly ovoid. The sexual stage was not produced.

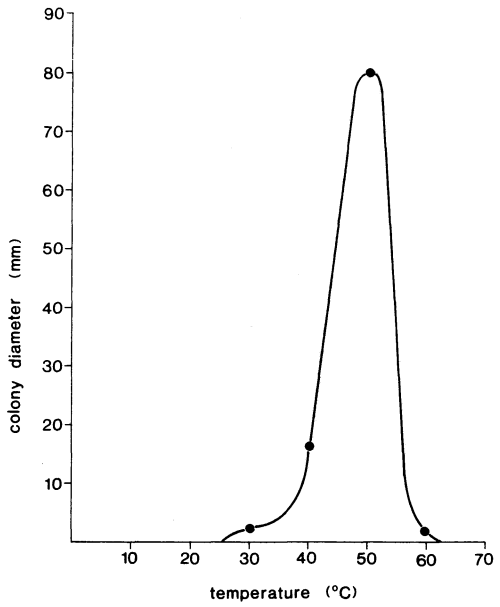

Fig. 1. Effect of temperature on growth of *P. dupontii* on YG agar after 7 days. Minimum growth temperature was 25°C, optimum was 50°C, and maximum near 60°C.

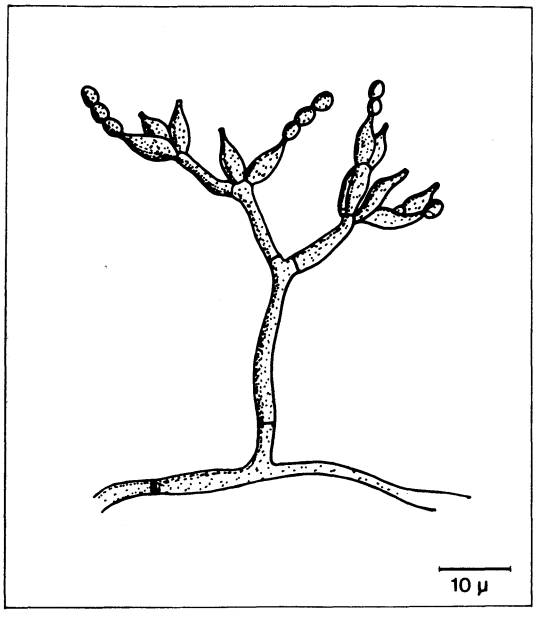

Fig. 2. *Talaromyces thermophilus*. Conidial state: *Penicillium dupontii* showing penicillus.

EXTRACELLULAR GLYCOSIDE HYDROLASE ACTIVITY

The culture filtrates obtained from growth in media containing the different carbon sources showed amylase, pectinase and cellulase activity (Table 1). Activity at 50° was greater than at 25°C.

TABLE 1. Amylase, polygalacturonate hydrolase and carboxymethylcellulase activities of culture filtrates, at 25 and 50°C

Carbon source	Starch		NaPP		CMC	
	25°	50°	25°	50°	25°	50°
Glucose	13	5	0	8	0	2
CMC	18	7	0	11	0	2
Sodium polypectate	15	4	0	12	13	1.8

No growth occurred in the culture fluid containing pectin as the sole carbon source.

AGAR-DIFFUSION ASSAY OF CELLULOLYSIS

P. dupontii did not form a zone of clearing in the medium containing acid-swollen cellulose. Growth was poor on this medium.

DISCUSSION

T. thermophilus was found to be a true thermophilic fungus (*sensu*. Cooney and Emerson 1964). One of the most critical and difficult distinctions to be made is that between thermophilism and thermodurism. Thermophilism is based on the genetic constitution of the organism which permits normal growth at these elevated temperatures and limits it at lower temperatures. In contrast, thermodury may result from genetic adaptation which permits fundamental processes to occur or limits the destruction of these processes at otherwise lethal temperatures. The ubiquity of most thermophilic fungi, which can be isolated from situations where the minimal temperature for growth would never be attained, is difficult to explain in terms of the obligate thermophily found in the laboratory. Tendler *et al.* (1967) showed that the minimum temperature for growth of several thermophilic fungi can be reduced by nutritional manipulations suggesting that obligate thermophily may be an artifact of the nutritional environment.

Little is known about amylase production in thermophilic fungi. A number of thermophiles have been shown to produce amylase (Fergus 1969b), and amylase production by *T. thermophilus* has been confirmed in these experiments.

Amongst the thermophilic fungi only *Sporotrichum thermophile* has been shown to be able to decompose pectin (Henssen 1957). Although *T. thermophilus* was unable to utilize pectin as the sole carbon source for growth, a pectate-degrading enzyme was elaborated in the three different carbon source media. There have been many reports of cellulase production by thermophilic fungi

and good cellulase activity as assayed by the cup-plate method was obtained from cultures growing on media containing all three carbon sources. Surprisingly, the medium containing sodium polypectate proved to be the best for cellulase production. Reese *et al.* (1950) suggested that the hydrolysis of CMC is due to an enzyme C_x which is distinct from the enzyme degrading native cellulase (C_1) and the cup-plate method used would only indicate C_x activity. *T. thermophilus* was shown to produce carboxymethylcellulase which is in disagreement with the results of Chang (1967) and Fergus (1969a). However, a considerable number of strains differing with respect to cellulase production are known to occur.

Tansey (1971) reported that *T. thermophilus* was unable to clear acid swollen cellulase and our results agree with this finding. While noting Fergus's (1969a) borderline results for C_x activity, Tansey concluded that *T. thermophilus* is not cellulolytic. Our findings do not substantiate this claim since our islate was definitely cellulolytic.

These studies have shown that the thermophilic fungus *T. thermophilus* has the ability to thrive at high temperatures and to utilise high molecular weight, polymeric, carbon sources at high temperatures - two important characteristics of successful colonizers of organic matter (Chang 1967). It seems probable, therefore, that fungi such as *T. thermophilus* are important in the decomposition of composts and in carbon recycling. These fungi may well prove to be useful for municipal solid-waste composting.

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